CFTR INHIBITORS AS POTENTIAL THERAPY TO REDUCE INTESTINAL FLUID LOSS IN CHOLERA AND OTHER SECRETORY DIARRHEAS

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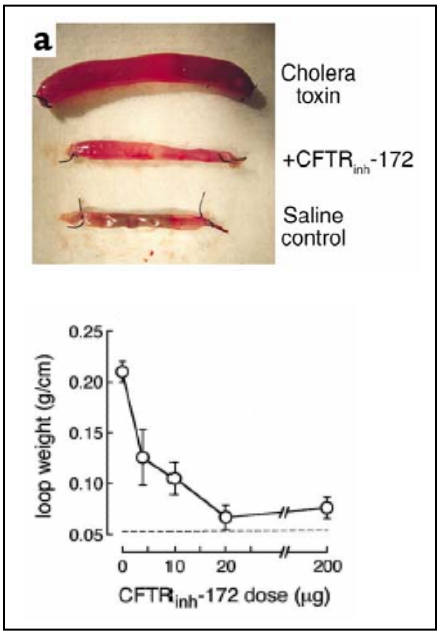
There is a need to develop a safe and effective treatment for secretory diarrhea induced by *Vibrio cholerae* and enterotoxigenic *E. coli* (ETEC). The molecular mechanism leading to diarrheas caused by these organisms is well understood. Both cholera and ETEC disease is typified by enterotoxin production which results in cAMP production. In addition, ETEC infection frequently results in liberation of stable toxin (ST) that activates production of cGMP. A cascade of events ensues, ultimately causing phosphorylation of the ABC transporter CFTR. Because this transporter is an integral chloride channel, phosphorylation increases chloride efflux that, in turn, actively drives sodium and water secretion into the intestinal lumen resulting in a form of diarrhea known as secretory diarrhea[1]. An analogous situation holds in cholera, where the toxin irreversibly activates the cAMP signaling cascade with attendant phosphorylation of CFTR. Activation of CFTR by ETEC and cholera toxin is directly related to the severity of the diarrhea seen in these two disease states. Accordingly, pharmacological inhibition of CFTR has been shown to be a strategy for the attenuation of secretory diarrheas in animal models of cholera and ETEC infection[2-4] and is therefore a strategy for the treatment of travelers diarrhea[5] (TD); a transient secretory diarrhea most commonly caused by infection with ETEC[6, 7]. CFTR is expressed on the apical surface of epithelial tissues including bronchial passages, pancreatic ducts, sweat ducts and the gastrointestinal tract[8]. Cystic fibrosis patients and animal models have decreased gastrointestinal secretory activity[9] and both CF patients and CFTR knockout mice do not develop diarrhea in response to heat stable enterotoxins[10].

Using high-throughput screening (HTS), Alan Verkman (University of California, San Francisco) has identified two classes of potent CFTR inhibitors with potential as anti-diarrheal therapy. The first class discovered was thiazolidinones and one of these is the small-molecule CFTR inhibitor, 3-[(3-trifluoromethyl)phenyl]-5-[(3-carboxyphenyl) methylene]-2-thioxo-4-thiazolidinone ($CFTR_{inh}$-172)[4]. The later compound class discovered was the glycine hydrazide class which, by electrophysiology studies, appears to block the CFTR ion channel on the lumen-facing surface[11]. The most potent compound from both these classes has potency in the low micromolar IC50 range with the value varying somewhat depending on the type of assay and manner in which it is executed.

Both of these compounds were originally discovered through the use of a cell-based FRT assay in which CFTR activity is pre-stimulated by addition of forskolin (cAMP agonist), IBMX (phosphodiesterase inhibitor and direct activator) and apigenin (flavone-type direct activator). The FRT cells co-expressed the Yellow Fluorescent Protein (YFP) -based Cl^- / I^- sensor YFP-H148Q that provided a quantitative fluorescence read-out of inhibition potency. After CFTR pre-stimulation and compound addition, cells were subjected to an inwardly-directed I^- gradient to drive I^- influx and produce decreasing fluorescence. These molecules were also assayed using the techniques of short circuit current measurements in Ussing chambers and whole cell patch clamp electrophysiology with various membrane and cell types. This provides another route to measure inhibitory potency of these compounds.

An *in vivo* model was used to test the compound's antidiarrheal efficacy in cholera toxin-induced diarrhea. Both intestinal fluid absorption and secretion were tested in a closed intestinal loop mouse model[12]. Intraperitoneal administration of $CFTR_{inh}$-172 at a dose that strongly inhibited cholera toxin-induced intestinal fluid secretion (20 μg) did not alter the rate of fluid absorption (measured at 30 min) compared to controls.

Both the thiazolidinone and glycine hydrazide CFTR inhibitor block cholera toxin-induced fluid secretion in closed intestinal loops. The figure below summarizes a $CFTR_{inh}$-172 dose-response study in mice in which a single dose of inhibitor was administered by intraperitoneal injection just after infusion of cholera toxin into closed intestinal loops. Similar results were seen for the glycine hydrazide compound. Basal intestinal fluid content was near zero as measured in non-cholera toxin injected loops. $CFTR_{inh}$-172 inhibited fluid accumulation in cholera toxin-injected intestinal loops by ~90%, with 50% inhibition at ~5 µg $CFTR_{inh}$-172. The duration of inhibition was measured as in the dose-response study, except that a single 20 µg dose of $CFTR_{inh}$-172 was administered at different times before or after cholera toxin. Good efficacy was seen for time points up to 3 hours before and after cholera toxin challenge. These experiments were carried out with both mice and rats and fluid accumulation was stimulated with both cholera toxin and heat stable ETEC toxin. In all cases the CFTR inhibitors tested reduced fluid accumulation.



The CFTR inhibitor $CFTR_{inh}$-172 is under development as a candidate antidiarrheal drug treatment. Toward that end, a range of development activities have been conducted including continued efficacy studies, single and multi-dose toxicology experiments, pharmacokinetic evaluation, distribution studies, formulation research, specificity evaluation, metabolism, bioanalytical and analytical method development, stability, manufacturing development, and solubility and permeability evaluation

Significant work remains to be done in the evaluation and development of CFTR inhibitors for the treatment of secretory diarrhea. Our work to date has been very encouraging and Active Pass continues to develop these molecules toward clinical trials, targeting the first clinical evaluation in 2006.

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